IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Clarence N. Ahlem, et al.
Application No.	: 10/607,035
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DECLARATION UNDER 37 C.F.R § 1.132

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Christopher L. Reading, declare as follows:

1. I am a co-inventor of the above-referenced patent application. I have been engaged in the evaluation and development of therapeutic agents and treatment methods for over 20 years, which includes 7 years of experience with preclinical and clinical development of steroid compounds. A summary of my resume is attached hereto. The following statements are based on the documents identified below, my knowledge of the experiments and results that are discussed below and/or my professional training and experience.

2. I have read U.S. patent No. 5,532,230 (the '230 patent), and I do not believe that the disclosure teaches or suggests the use of any of the presently claimed compounds for treating inflammation or atopic asthma. I also do not believe that the '230 patent provides a basis for one of ordinary skill in the art to

have a reasonable expectation that the presently claimed compounds could successfully be used to treat inflammation or atopic asthma. The '230 patent at columns 8-9, defines the compounds the patent is concerned with. The definition of the formula I structure at columns 8-9 includes tens of millions of compounds. The compounds as defined do not include any of the compounds that are recited in the claims and the definition of R^2 expressly states that the 16-position is unsubstituted when the 17-position is substituted with a hydroxyl group. This definition therefore excludes the molecules that the present claims recite. The claims of the '230 patent contain this same limitation. At column 7, lines 8-15, the '230 patent suggests DHEA analogs that are modified at the 2-, 4-, 6- or 7-positions. At column 9, lines 6-10, the '230 patent suggests DHEA analogs that are modified at the 2-, 4- or 7-positions. Had such compounds been intended, the '230 patent would have defined the chemical structure that way. Since this definition expressly excludes other structures, my opinion is that the '230 patent does not suggest any substitution at the 16-position when a hydroxyl group is present at the 17-position. Given that, one of ordinary skill in the art would not have a reasonable expectation that the use of such compounds could successfully be used to treat any inflammation condition or atopic asthma.

3. There are other considerations in addition to that discussed in paragraph 2 of why the '230 patent does not teach or suggest the use of the presently claimed compounds for treating inflammation or atopic asthma. The '230 patent does not disclose or suggest any compound that has a substituent at the 3-position in the α-configuration and/or a substituent in the 16-position that is in the β-configuration, which many of the presently claimed compounds have. The discussion at column 15, lines 8-27 states that DHEA, which has a ketone (=O) at the 17-position is the active compound among those tested. This means that a compound such as 3α,16β,17β-trihydroxyandrostane, discussed further below, would not be expected to be active because such compounds cannot convert to DHEA *in vivo*. Given these considerations, there is again no reason for one of ordinary skill in the art to consider using the presently claimed compounds

to treat inflammation or atopic asthma, nor is there any basis for one of ordinary skill in the art to have a reasonable expectation that such compounds would be effective.

4. When two representative compounds within the scope of the present claims were characterized for their capacity to treat lung inflammation in animals, they were found to be unexpectedly potent and the nature of the biological response was also unexpected. Three compounds, 3β,16α-dihydroxy-17-oxoandrostane, 3α,16β,17β-trihydroxyandrostane and 3α,16α,17α-trihydroxyandrostane were used to treat inflammation in animals essentially as described in a published protocol (D. Auci et al., *Ann. New York Acad. Sci.* 1051:730-742 2005, newly cited). Five to 8 week old CD1 male mice (Charles River, Calco, Italy) were used for the study. The animals were housed in a controlled environment and provided with standard rodent chow and water. Animal care was in compliance with applicable regulations on protection of animals. Mice were allocated into one of the following groups: (1) mice treated with 2% carrageenan-λ in saline (carrageenan-λ treated control group), (2) mice treated with 0.1 mg, 0.01 mg or 0.001 mg 3β,16α-dihydroxy-17-oxoandrostane by subcutaneous (s.c.) injection 24 h and 1h before carrageenan-λ administration, (3) mice treated with 0.1 mg, 0.01 mg or 0.001 mg of 3α,16α,17α-trihydroxyandrostane by s.c. injection 24 and 1 h before carrageenan; (4) mice treated with 0.1 mg, 0.01 mg or 0.001 mg 3α,16β,17β-trihydroxyandrostane by s.c. injection 24 h and 1 h before carrageenan-λ administration; (5) mice treated with vehicle (0.1% carboxymethylcellulose, 0.9% saline, 2% tween 80, 0.05% phenol) s.c. 24h and 1 h before carrageenan-λ administration; (6) mice treated with rabbit anti-mouse polyclonal anti-TNF-α antibody (200 μg) given as an intraperitoneal bolus 24 h and 1h before carrageenan-λ administration (positive control group); and (7) sham-operated mice that were not treated with carrageenan-λ. Each group consisted of 10 mice. All treatments were given in a final volume of 100 μL. Lung (pleural cavity) inflammation was induced as

follows. The mice were anaesthetised with isoflurane and a skin incision was made at the level of the left sixth intercostal space. The underlying muscle was dissected and either 0.1 mL saline (control) or 0.1 mL saline containing 2% λ-carrageenan was injected into the pleural cavity. The carrageenan-λ is a potent inducer of inflammation, which is manifested in this protocol by accumulation of fluid and neutrophils in the pleural cavity. The incision was closed with a suture and the animals were allowed to recover. At 4 h after the injection of carrageenan-λ, the animals were euthanized by exposure to CO_2. The chest was carefully opened and the pleural cavity rinsed with 1 mL of saline solution containing heparin (5 U/mL) and indomethacin (10 μg/mL). The exudate and washing solution were removed by aspiration and the total volume measured. Any exudate contaminated with blood was discarded. The amount of exudate was calculated by subtracting the injected 1 mL volume from the total pleural cavity volume that was recovered. The neutrophils in the exudate were suspended in phosphate-buffer saline and counted with an optical microscope in a Burker's chamber after Trypan Blue staining. The results were analysed by one-way ANOVA followed by a Bonferroni *post-hoc* test for multiple comparisons. A *p*-value less than 0.05 was considered significant. For statistical analysis each group was compared to the control group of mice that were challenged with carrageenan-λ and received no other treatment.

5. All of the mice that were challenged with carrageenan-λ and were left untreated developed an acute pleurisy, producing turbid exudate and increased pleural numbers of neutrophils. The increase in volume exudates and numbers of leukocytes in the pleura of the mice treated with the vehicle was similar to that observed in the control mice that were challenged with carrageenan-λ and received no treatment. Relative to these two groups of control mice, group 3 animals treated with 3β,16α-dihydroxy-17-oxoandrostane showed a significant reduction in the number of neutrophils in the pleura the volume of pleural exudates at the 0.1 mg 0.01 mg doses, while the lower 0.001 mg dose was inactive. The volume of pleural exudate at the 0.1 mg dose in the treated with

3β,16α-dihydroxy-17-oxoandrostane was significantly reduced, but not at the lower 0.01 mg and 0.001 mg doses. Animals treated with 3α,16α,17α-trihydroxyandrostane showed a significant reduction in the number of neutrophils in the pleura at the 0.1 mg and 0.01 mg doses. Treatment with 3α,16β,17β-trihydroxyandrostane also showed a significant reduction in the number of neutrophils in the pleura at the 0.1 mg and 0.01 mg doses. The potency of 3α,16α,17α-trihydroxyandrostane and 3α,16β,17α-trihydroxyandrostane were similar to that observed with the polyclonal anti-TNF-α antibody control, while 3β,16α-dihydroxy-17-oxoandrostane was less potent. The table below describes the number of neutrophils from the treated animal groups relative to untreated control animals that were exposed to carrageenan-λ, but not treated with anything else (negative control group). The neutrophil number for the negative control group was set at 100% and other groups were compared to this. The group of animals that were treated with anti-TNF-α antibody (positive control group) had 29% of the number of neutrophils the negative control group had, which indicates that the antibody had an antiinflammatory effect against the carrageenan-λ exposure. The vehicle control group did not have a significantly reduced number of neutrophils (91%) compared to the negative control group, which shows no significant antiinflammatory effect due to the vehicle alone.

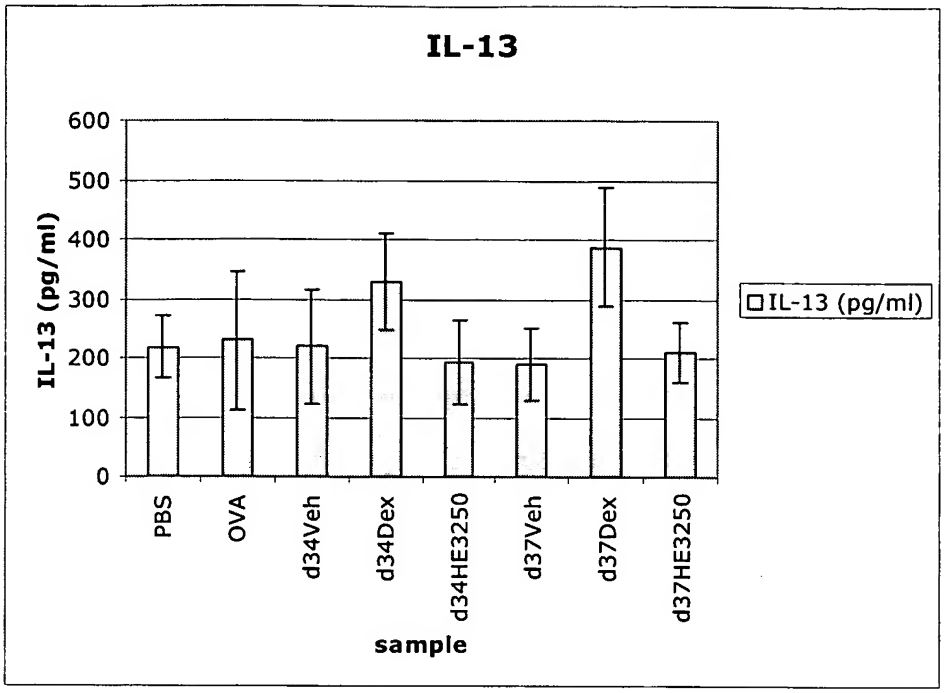
3β,16α-dihydroxy-17-oxoandrostane		3α,16α,17α-trihydroxyandrostane		3α,16β,17β-trihydroxyandrostane	
0.001 mg	97%	0.001 mg	103%	0.001 mg	95%
0.01 mg	73%	0.01 mg	45%	0.01 mg	50%
0.1 mg	73%	0.1 mg	30%	0.1 mg	42%

6. In another analysis, the compound 3α,16α,17α-trihydroxyandrostane was found to have biological properties that would made the compound superior as an agent to treat an inflammation condition such as atopic asthma. Specifically, the use of the compound was not accompanied by a rebound in IL-13, which is a known side effect of antiinflammatory glucocorticoid compounds such as dexamethasone. To the best of my knowledge, this lack of an IL-13

rebound was not known for any antiinflammatory compound. The protocol was as follows. Specific pathogen free, female BALB/cAnNCrl (6 weeks of age) were obtained from Charles River (Sulzfeld, Germany). Mice were allowed to settle for a week before random assignment into treatment groups. Mice were maintained under barrier conditions at a mean temperature of 23 ± 2°C, 50-55% humidity, and a 12 hour light/dark cycle. Drinking water and standard laboratory food pellets were provided *ad libitum*.

7. Antigen (ovalbumin) naïve mice were subcutaneously injected into the right hind footpad with 50 μL of a freshly prepared mixture of 0.01, 0.3, or 3.0 mg of 3α,16α,17α-trihydroxyandrostane together with a sub-sensitizing dose (10 μg) of trinitrophenyl-ovalbumin (TNP-OVA) diluted in vehicle (0.1% carboxymethylcellulose, 0.9% saline, 2% tween 80, 0.05% phenol) on day 0. Injection was performed using a 25-gauge needle in toe-to-heel direction. One week later, mice were euthanized by cervical dislocation and the popliteal lymph node (PLN) was excised and separated from adherent fatty tissue. The PLN was isolated in ice-cold RPMI 1640 with Glutamax (Life Technologies, Breda, the Netherlands) supplemented with 10 % fetal calf serum and 2% penicillin-streptomycin. Single-cell suspensions were prepared, washed (1000 rpm, 4°C), resuspended in 1 mL medium, counted using a Coulter counter (Coulter Electronics, Luton, UK) and adjusted to $2.5*10^6$ cells/ml. IL-13 was measured in the PLN cells by flow cytometry.

8. As shown in the diagram below, the 3α,16α,17α-trihydroxyandrostane did not generate an IL-13 increase that was observed with animals that had been treated with dexamethasone (dex).



9. I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 4/21/06 By: Christopher L. Reading

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Professional credentials and experience

- Ph.D. in Biochemistry, U.C. Berkeley, 1977
- Postdoctoral Fellowship in Tumor Biology, U.C. Irvine, 1978-1980
- Faculty, M.D. Anderson Cancer Center, 1980-1993
  Tenured Associate Professor of Medicine, Department of Hematology
  Stem Cell Transplantation and Gene Therapy
  Four granted patents in bispecific monoclonal antibodies and devices
- SyStemix, Inc. 1993-1998
- Vice President for Product and Process Development 1996-1998
  Senior Management team involved in sale of SyStemix, Inc. to Novartis
  Senior Director of Cellular Purification 1993-1996
  IND for autologous stem cell isolation for cancer
  IND for in utero transplantation
  IND for stem cell gene therapy for HIV
- Novartis Biotechnology Development and Production 1997-1998
- Cell and Gene Therapy Strategy
  Immune Cell Therapy Strategy
  Technical Analyst for Mergers and Acquisitions in Cell and Gene Therapy
  Technical Analyst for Intellectual Property in Cell and Gene Therapy
  Technical Analyst for Business Development and Licensing
  REV123 HIV Gene Therapy International Project Team
  GTI/SyStemix Technical Research and Development Integration Team
- Hollis-Eden Pharmaceuticals 1998-Present
- Vice President for Scientific Development
  IND for 16α-bromoepiandrosterone treatment of HIV
  IND for 3β, 7β, 17β-androstenetriol in vaccination of the elderly
  International clinical trial development for HIV, Malaria, HBV, HCV
  Established collaborations in South Africa, Thailand, Singapore and Australia
  Frequent presentations to Investment Bankers and Wall Street
- International Scientific Reputation 1977-2000
- 30 National and International Scientific Presentations
  77 publications in peer-reviewed journals
  18 invited journal articles
  20 invited book chapters
  National Science Foundation Advisory Committee for SBIR Grants

Editorial Board, Journal Biological Response Modifiers
Editorial Board, Molecular Biotherapy
Peer-reviewed Grants and Contracts totaling over $2 Million
Consultant to Government Agencies and Private Corporations

Exemplary Published Articles in Peer-Reviewed Journals

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